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Silahtaroglu, Asli; Stenvang, Jan

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MicroRNAs, epigenetics and disease

Asli Silahtaroglu*[1] and Jan Stenvang†

**Wilhelm Johannsen Centre for Functional Genome Research, Department of Cellular and Molecular Medicine, University of Copenhagen, Blegdamsvej 3, DK-2200, Copenhagen N, Denmark, and †Department of Veterinary Disease Biology, Faculty of Life Sciences (LIFE), University of Copenhagen, Dyrlægevej 88, 1870 Frederiksberg C, Denmark*

Abstract

Epigenetics is defined as the heritable chances that affect gene expression without changing the DNA sequence. Epigenetic regulation of gene expression can be through different mechanisms such as DNA methylation, histone modifications and nucleosome positioning. MicroRNAs are short RNA molecules which do not code for a protein but have a role in post-transcriptional silencing of multiple target genes by binding to their 3′ UTRs (untranslated regions). Both epigenetic mechanisms, such as DNA methylation and histone modifications, and the microRNAs are crucial for normal differentiation, development and maintenance of tissue-specific gene expression. These mechanisms also explain how cells with the same DNA content can differentiate into cells with different functions. Changes in epigenetic processes can lead to changes in gene function, cancer formation and progression, as well as other diseases. In the present chapter we will mainly focus on microRNAs and methylation and their implications in human disease, mainly in cancer.

[1]*To whom correspondence should be addressed (email asli@sund.ku.dk).*

Introduction to microRNAs

miRNAs (microRNAs) are a class of short (approx. 22 nt) endogenous non-coding RNAs that act as post-transcriptional regulators of gene expression. According to the September 2009 release of the microRNA database mirbase (http://www.mirbase.org/) there are 10883 miRNA entries from vertebrates, insects, plants and viruses discovered either by cloning or bioinformatics [1]. Among them 721 are detected in humans. The first member of the miRNA family, lin-4, was originally identified in *Caenorhabditis elegans* as a developmental timing regulator [2]. miRNAs play fundamental roles in the control of many biological processes such as growth, development, differentiation, proliferation and cell death [2,3]. They perform these functions by repression of their target genes. Each miRNA may target several hundred mRNAs and more than 60% of the mRNAs are predicted to have a miRNA-binding site in their 3′ UTR (3′ untranslated region). The huge number of miRNAs identified and evidence accumulated over the years indicate that a vast number of normal and pathological mechanisms are controlled by miRNA-mediated regulatory networks (reviewed in [4,5]).

Genomic organization, biogenesis and function

miRNAs can be intergenic, intronic or exonic. Intergenic miRNAs have either their own promoters (monocistronic) or share the same promoter (polycistronic), whereas the intronic miRNAs are present either singly or in clusters using the promoter of their host gene. miRNAs are transcribed in the nucleus by RNA polymerase II. They are 5′ capped and 3′ polyadenylated. The maturation of miRNAs requires two endonucleolytic cleavage steps by RNase III-like enzymes: Drosha and Dicer. Following transcription, Drosha processes the primary miRNA transcript (pri-miRNA), which can be several kilobases long, into a 60–100 nt hairpin structure named the precursor-miRNA (pre-miRNA). Pre-miRNAs are folded into mini-helical structures to be recognized by exportin-5, the nuclear export factor carrying the pre-miRNAs from the nucleus to the cytoplasm. In the cytoplasm, the pre-miRNA hairpin is cleaved at the loop end by Dicer, thereby creating a 22 nt RNA duplex comprising the mature miRNA guide strand and the miRNA* passenger strand. The mature miRNA is loaded into the RISC (RNA-induced silencing complex), whereas the passenger strand is degraded (Figure 1). The exact details of the miRNA biogenesis mechanism are still to be investigated, and much less is known about the mechanisms regulating the expression of miRNAs. Recent studies point out that not all miRNAs are created by the same mechanisms. After being loaded into the RISC complex mature miRNAs are directed to their binding sites in their target mRNAs. In broad terms, this binding leads to repression of mRNA translation by one of the following mechanisms: translational block by folding the mRNA in an inactive steric conformation, deadenylation and destabilization of the

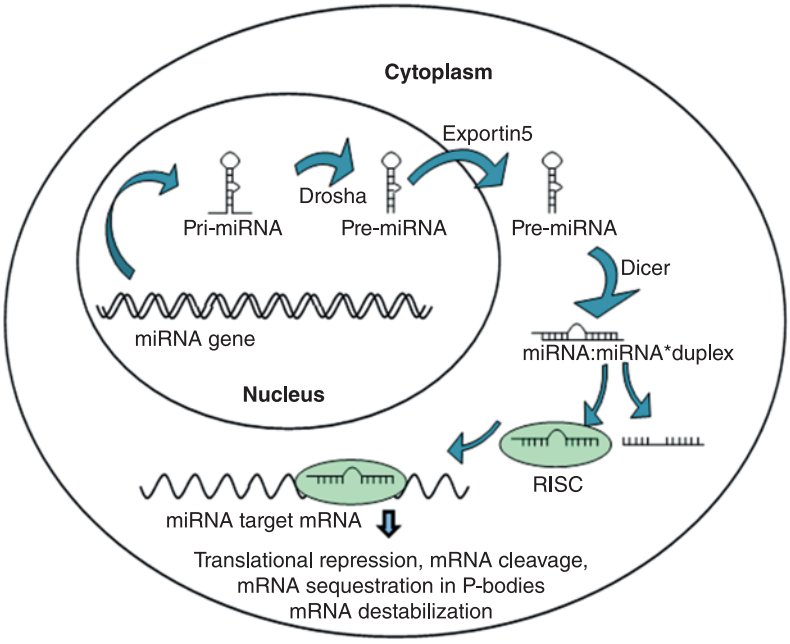

Figure 1. Schematic representation of the process from transcription of a miRNA gene in the nucleus to mature regulatory miRNA binding to target mRNA in the cytosol

Upon binding to the target mRNA the process may be negatively regulated by different mechanisms, i.e. translational repression, mRNA destabilization, mRNA cleavage or sequestration in P-bodies.

mRNA, cleavage of the mRNA or sequestration in P-bodies (processing bodies) (reviewed in [4,6,7]).

miRNAs regulate important biological processes

For many biological functions it is very important to have the miRNA expression in balance. Developmental timing, differentiation, organogenesis, cell proliferation, apoptosis, differentiation of embryonic stem cells, limb development, synaptic development and plasticity, skin differentiation, cardiogenesis, normal immune function and regulation of insulin secretion in the pancreas are some of the biological functions where miRNAs play a crucial role (reviewed in [7]).

miRNA and disease

In the last decade it has become clear that aberrant miRNA deregulation and expression is observed in most human malignancies, although it is often not clear whether this deregulation is the cause or the effect of the disease. Some of the most investigated malignancies are cancers, dysfunctional heart conditions, metabolic diseases and viral infections. Very recently a knowledge-base on

Table 1. Overview of various miRNAs that are either down-regulated or up-regulated in human tumour tissue

AML, acute myeloid leukaemia; CLL, chronic lymphocytic leukaemia.

miRNA	Tumour tissue	Changed expression in tumours
Let-7 family	Lung, breast, prostate, colon, gastric, ovary, CLL	Down-regulated
miR-101	Prostate, liver and bladder	Down-regulated
miR-122a	Liver	Down-regulated
miR-143	Colon, breast, lung, cervix, B-cell	Down-regulated
miR-145		
miR-15a	CLL, prostate, pancreas and multiple myeloma	Down-regulated
miR-16-1		
miR-155	CLL, Burkitt's lymphoma, lung, breast, pancreas and colon	Up-regulated
miR-221	Leukaemia	Down-regulated
miR-222		
miR-29 family	CLL, AML, breast, colon and lung	Down-regulated
miR-34 family	Pancreas, colon, breast and liver	Down-regulated
miR-372	Testis	Up-regulated
miR-373		
miR-17-92 cluster	Lymphomas, breast, lung, colon, stomach and pancreas	Up-regulated
miR-106b-93-25 cluster	Gastric, colon, prostate and neuroblastoma	Up-regulated
miR-21	Glioblastoma, ALM, CLL, Burkitt's lymphoma, breast, colon, pancreas, lung, prostate, liver and stomach, cervix, head and neck cancers	Up-regulated
miR-221	CLL, thyroid, liver and glioblastoma	Up-regulated
miR-222		

the aberrant expression of miRNAs in various diseases was introduced [8]. In cancers, miRNAs can act either as oncogenes or tumour suppressors, and a multitude of papers have investigated the differential expression of miRNAs in tumour tissues and their function in cancer cells and metastatic potential (see Table 1 for an overview). Many miRNAs act as tumour suppressor genes and they are frequently silenced in cancers. However, the underlying mechanism for this is less clear. One explanation is epigenetic silencing of miRNA genes and this has now been described in various cancers for several miRNAs.

Mechanisms such as change in turnover rate or DNA copy number could be other reasons for differential expression of miRNAs which need to be further investigated [9,10].

Introduction to miRNA and epigenetics

Epigenetic phenomenons such as DNA methylation of CpG islands in promoter regions of genes and histone modifications are well known to regulate gene expression. The major epigenetic changes in cancer are aberrant DNA hypermethylation of tumour suppressor genes, global genomic DNA hypomethylation and disruption of the histone modification patterns.

Like classical protein coding genes, miRNA genes are also subject to epigenetic regulation and miRNAs can also regulate various components of the epigenetic machinery. For a detailed review, see [11].

Epigenetic regulation of miRNA expression in cancer

Several miRNAs are down-regulated in cancer and act as *bona fide* tumour suppressor genes, and therefore these miRNAs are obvious candidates for epigenetic silencing. High-quality papers have been published on the impact of epigenetic regulation of miRNA genes with regard to cell proliferation, apoptosis, tumour suppressor or oncogenic effects both in cell culture systems, *in vivo* models and in various human primary tumours. The epigenetic status has also been correlated with metastatic status and with survival of cancer patients, which will be the focus of this part of the chapter. An up-to-date overview is presented in Table 2.

The concept of controlling miRNA expression by epigenetic mechanisms may be as widespread as for protein coding genes, since half of the miRNA genes are associated with CpG islands and miRNA gene methylation is detected with high frequency in normal and malignant cells [12].

A common approach to identifying epigenetically regulated miRNAs has been to treat cancer cells with inhibitors of DNA methylation (e.g. 5-azacytidine) and/or histone deacetylases [e.g. PBA (4-phenylbutyric acid) or TSA (trichostatin A)] and compare miRNA expression to that in untreated cells.

This approach was applied in the first two high-impact publications in this field from 2006, which identified a number of epigenetically regulated miRNAs in breast and bladder cancer cells [13,14].

It has turned out that epigenetic regulation of miRNA expression is a common hallmark in human cancers and that epigenetic tags are associated with metastatic status and clinically relevant endpoints, such as disease-free survival and overall survival, thereby suggesting their use as biomarkers in cancer detection, prognosis, monitoring and predicting response to treatment.

In CRC (colorectal cancer), epigenetically regulated miRNAs have been indentified in both cell lines and in tumour tissues. The expression of miR-342 was found to be regulated by CpG island methylation. Interestingly,

Table 2. Epigenetically controlled miRNAs in cancer

miRNA	Epigenetic silencing mechanism	Cancer	Identified target	*In vitro*/ *in vivo*	Consequence of epigenetic silencing	Biomarker potential	Reference
miR-124a	Methylation and histone modification	ALL	CDK6	*In vitro and in vivo*	Abnormal proliferation of ALL cells	Hypermethylation associated with higher relapse rate and mortality rate	[24]
miR-124a	Methylation	Gastric		*In vivo*		Methylation of miR-124a is increased by *H. pylori* infection	[42]
miR-9 miR-129 miR-137	Methylation (chromatin modification)	Colorectal		*In vitro*		Hypermethylated in CRC tumours compared with normal colonic mucosa.	[19]
				In vivo		Methylation of miR-9-1 is associated with lymph node metastasis	
Let-7a-3	Methylation	Colorectal		*In vitro*	Suppresses tumour phenotype	Hypomethylated in lung adenocarcinomas	[43]
				In vivo			
miR-203	Methylation	Haematopoietic tumours	ABL1	*In vitro*	Promotes tumour cell proliferation	Methylated in human leukaemias	[44]
			BCR-ABL1	*In vivo*			
miR-1-1	Methylation (histone modification)	Liver	FOXP1	*In vitro*	Promotes cell growth, replication potential and clonogenic survival		[45]

			MET HDAC4	*In vivo*			
miR-223	Methylation (chromatin remodelling)	AML		*In vitro*	Leukaemia differentiation block		[46]
				In vitro			
miR-124	Methylation	Hepatocellular carcinoma (HCC)	miR-124: CDK6, vimentin, SMYD3, IQGAP1	*In vitro*	Promotes HCC cell growth (124 and miR-203)	Tumour-specific methylation for miR-124 and miR-203	[47]
miR-203 miR-375			miR-203: ABCE1	*In vivo*			
miR-342	Methylation of the host gene EVL	Colorectal		*In vitro*	Anti-apoptotic	Increased methylation in CRC adenocarcinomas	[15]
				In vivo			
miR-181c	Methylation	Gastric colorectal	NOTCH4 KRAS		Promotes cell growth		[48]
miR-9-3	Methylation	Breast	p53-related apoptotic pathway	*In vitro*	Promotes cell proliferation		[49]
miR-129-2	Histone modification Methylation	Endometrial	SOX4	*In vitro*	Promotes cell proliferation	Hypermethylated in tumours correlated with a poor overall survival	[50]
	Histone modification			*In vivo*			
miR-21	Methylation	Ovary		*In vitro*			[51]

(*Continued*)

Table 2. (*Continued*)

miR-203							
miR-205							
miR-34b	Methylation	Oral	miR-137: CDK6	*In vitro*	miR-137 and miR-193a: promotes cell growth	Down-regulated through tumour-specific hypermethylation	[52]
miR-137			miR-193a: E2F 6	*In vivo*			
miR-193a							
miR-203							
miR-107	Methylation (histone TSA treatment)	Pancreatic	CDK6	*In vitro*	Promotes cell proliferation		[53]
miR-9-1	Methylation	Breast		*In vitro*		Hypermethylation of miR-9-1 in primary tumours	[18]
miR-124a3				*In vivo*			
miR-148							
miR-152							
miR-663							
miR-34a	Methylation	Prostate	CDK6	*In vitro*	Anti-apoptotic/oncogenic	Methylated in primary prostate carcinomas and primary melanoma	[54]
		Breast Lung Colon Kidney Bladder		*In vivo*			

		Pancreas Melanoma					
Let-7a-3	Methylation	Ovarian		*In vivo*		Methylated in ovarian cancer and associated to improved survival	[22]
miR-9 miR-34b/c miR-148a	Methylation	CRC	C-MYC	*In vitro*	miR-34b/c and miR-148a: promote mobility, tumour growth and metastasis formation	Hypermethylation is associated with lymph node metastasis	[55]
		Melanoma Head and neck Lung Breast	E2F3 CDK6 TGIF2	*In vivo*			
miR-124a	Methylation	Colon	CDK6	*In vitro*		Hypermethylated in CRC tumours	[16]
		Breast Lung Leukaemia Lymphoma Neuroblastoma Sarcoma		*In vivo*			
miR-370	Methylation	Cholangiocarcinoma	MAP3K8	*In vitro*	Promotes cell growth		[56]
				In vivo			

(*Continued*)

Table 2. (*Continued*)

miR-193b	Methylation	Prostate		*In vitro*	Promotes cell growth		[57]
				In vivo			
miR-9-1/2/3	Methylation	ALL		*In vitro*		Hypermethylation predicts DSF and OS	[23]
miR-10b miR-34b/c miR-124a1/2/3 miR-132 miR-196b miR-203 miR-212	Histone modification			*In vivo*			
miR-127 and others	Methylation	Prostate	BCL6	*In vitro*		Heavily methylated in both normal and tumour tissue	[13]
	Histone modification	Bladder Colon		*In vivo*			
miR-126	Methylation	Prostate		*In vitro*			[58]
	Histone modification Intronic miRNA regulated by methylation of host gene (EGFL7)	Bladder		*In vivo*			
miR-512-5p	Methylation	Gastric	MCL-1	*In vitro*	Anti-apoptotic		[59]

	Histone modification					
miR-27a/b	Histone modification	Breast	ZBTB10/RINZF RYBP/DEDAF	*In vitro*		[14]
miR34b/c	Methylation	CRC		*In vitro* *In vivo*	Hypermethylated in 90% of tumours	[17]
C19MC cluster	Methylation	Gastric		*In vitro*		[60]
miR-141	Methylation	Breast		*In vitro*		[61]
miR-200c	Histone modification	Prostate				
Various	Methylation (approx. 50% of miRNA genes associated with CpG islands)	Cervix				[12]
		Colon				
Various (miRNA cluster at Dlk1-Gtl2 Domain)	Methylation	Ovarian	Various (microarray analysis)	*In vitro*	Down-regulation of miRNAs located at *Dlk1-Gtl2* domain is associated with higher tumour proliferation and shorter patient survival.	[21]
		Breast Colon		*In vivo*		

methylation of miR-342 may be specific to CRC, since *in vitro* studies employing 40 non-CRC cell lines only found partial methylation in a single cell line. Analysis of tissue indicated that methylation of miR-342 may be an early event in CRC since methylation was detected in 86% of CRC adenocarcinomas and in 67% of adenomas [15]. Comparison of normal and colon cancer tissues has shown that miR-124a is hypermethylated in 75% of the tumours (n=56) [14]. Methylation of miR-124a was also found in tumours from the lungs (48%, n=27) and breast (32%, n=22), but not in neuroblastomas or sarcomas [16]. Analysis of primary CRC tumours (n=111) and adjacent normal colon (n=17) found that miR-34b/c was methylated in 90% of the primary CRC tissues and very limited methylation was found in the normal mucosa [17].

In primary breast cancer specimens, aberrant hypermethylation has been shown for miR-9-1, miR-124a3, miR-148, miR-152 and miR-663 in 34–86% of cases in a series of 71 primary human breast cancer specimens [18]. The miR-9-1 gene is hypermethylated in pre-invasive intraductal lesions, suggesting that hypermethylation of miR-9-1 is an early and frequent event in breast cancer development.

Two reports have associated methylation of miRNA genes with metastatic status of cancer patients.

Bandres et al. [19] identified five down-regulated miRNAs in primary CRC, which were located in the vicinity (<1000 bp) of a CpG island. Methylation status for three of these were analysed in primary CRC samples and adjacent normal tissue, and miR-9-1, miR-129-2 and miR-137 were methylated in 56% (n=36), 91% (n=34) and 100% (n=31) of primary CRC cases respectively. Methylation of miR-9-1 was totally absent in histological normal mucosa and methylation was more frequent in stage III and IV compared with stage I and II. Importantly, methylation status of miR-9-1 was associated with regional nodal invasion, vascular invasion and metastasis in a group of 32 patients (16 non-methylated and 20 methylated).

In a recent report, direct relation of miRNA hypermethylation and metastasis was explored [20]. Cell lines established from lymph node metastasis were treated with 5-azacytidine and the miRNA expression relative to untreated cells was investigated. These experiments identified 16 hypermethylated and up-regulated miRNAs, located in the proximity of a CpG island. Comparison with methylation status of non-cancerous tissues further reduced the number of miRNAs displaying cancer-specific CpG island hypermethylation. The selected miRs – miR-148a, miR-34b/c, and miR-9-1/2/3 – were tested *in vitro* and *in vivo* for their potential involvement in metastasis. Re-introduction of miR-34b/c and miR-148 into a metastatic carcinoma cell line, which is hypermethylated and silenced for miR-34b/c and miR-148 expression, reduced the migratory capability of the cancer cells. Likewise, experiments with nude mice showed that re-introduction of miR-34b/c and miR-148 caused reduced tumour growth and diminished metastatic potential of the metastatic carcinoma

cell line. A collection of primary tumour samples (n=278) from various tumour types were analysed and hypermethylation was undetectable in the corresponding normal tissue. Notably, hypermethylation of miR-34b/c, miR-148 and miR-9-3 in primary tumours was significantly associated with those tumours that were positive for metastatic cancer cells in the corresponding lymph nodes (n=207).

In a report focusing on ovarian cancer, eight miRNAs (miR-337, miR-368, miR-376a/b, miR-377, miR-410, miR-432, miR-495) located in the chromosome 14 miRNA cluster (*Dlk1-Gtl2* domain) were identified as potential tumour suppressor genes regulated by DNA methylation [21]. An expression signature separated late-stage ovarian cancers (n=73) into two distinct clusters. Patients belonging to the cluster with low expression of the eight miRNAs displayed higher tumour proliferation and had shorter 5-year survival. Analysis of other cancer types indicated that down-regulation of the chromosome 14 miRNA cluster may be an event common to many human epithelial tumours.

The let-7a-3 gene is located in a CpG island and its methylation status was analysed in 214 malignant tumours: no correlation between disease stage and tumour grade was detected [22]. Although the disease-free survival was not associated with methylation of let-7a-3, the patients with low let-7a-3 methylation (n=138) had significantly worse overall survival than those with high methylation (n=67).

Two reports from the same laboratory have analysed epigenetic regulation of miRNA expression in ALL (acute lymphoblastic leukaemia) and associated it with clinical outcome.

In ALL-derived cell lines, analysis of histone modifications around CpG islands located in the 5´ UTR of miRNA genes identified 13 miRNA candidate genes for epigenetic silencing: miR-9-1/2/3, miR-10b, miR-34b/c, miR-124a1/a2/a3, miR-132, miR-196b, miR-203 and miR-212 [23]. Methylation of at least one in 13 miRNA was found in 65% (n=353) of the ALL human tumours and was a strong and independent negative prognostic marker for disease-free survival and overall survival.

In ALL patients, miR-124 is regulated by CpG island hypermethylation and histone modifications, and re-introduction of miR-124a severely reduced tumorigenicity of ALL cells in a xenograft mouse model [24]. The miR-124a methylation status was analysed in 353 ALL patients and hypermethylation was found in 59%; this correlated with decreased expression of miR-124a. Furthermore, hypermethylation significantly correlated with higher relapse and mortality rates, and multivariate analysis showed that miR-124a is an independent prognostic factor for both disease-free survival and overall survival.

Taken together, these results show that DNA demethylation and HDAC (histone deacetylase) inhibition can activate expression of miRNAs and further large-scale clinical investigations are clearly warranted.

miRNAs as regulators of epigenetic processes

As well as being regulated by epigenetic mechanisms, miRNAs also play a role in controlling the chromatin structure by post-transcriptional regulation of chromatin-modifying enzymes (reviewed in [11,25]). Among the predicted human miRNA target genes there are a number of genes involved in epigenetic regulation, such as the methyl CpG-binding proteins, HMTs (histone methyltransferases), chromodomain-containing proteins and HDACs [26]. This subset of miRNAs, which directly or indirectly regulate the expression levels of effectors of the epigenetic processes, have been termed 'epi-miRNAs' [11]. Aberrant regulation of miRNA expression plays an important and direct role in the aberrant epigenetic silencing of tumour suppressor genes by DNA methylation in human cancers (see Table 3).

DNA methylation patterns are laid down during development by DNMT3a and DNMT3b (where DNMT is DNA methyltransferase), whereas maintenance during replication is facilitated by DNMT1. The first direct link between a miRNA and the DNMTs was established between the miR-29 family (miR-29a/b/c) and DNMT3a and DNMT3b, and other miRNAs such as miR-148 and miR-143 have also been indicated as regulators of the methylation enzymes. In non-small cell carcinoma of the lung, miR-29 is down-regulated, whereas the DNMT3A and DNMT3B expression is increased. Re-expression of miR-29 is shown to disrupt the *de novo* DNA methylation and caused general hypomethylation, leading to expression of tumour suppressor genes that are silenced by methylation, which resulted in apoptosis in cancer cells both *in vitro* and *in vivo*. This study indicated that miR-29 regulates the DNMT3 genes in lung cancer and revealed a new mechanism whereby the miRNAs indirectly regulate the gene expression through direct regulation of epigenetic mechanisms [27]. Another group has shown that overexpression of *miR-29b* in AML (acute myeloid leukaemia) cells resulted in marked reduction of DNMT1, DNMT3A and DNMT3B at both the RNA and protein levels. They concluded that the expression of *miR-29b* promoted DNA hypomethylation not only through direct targeting of DNMT3a and DNMT3b, but also by decreasing the DNMT1 expression indirectly via down-regulation of *Sp1*, a known transactivating factor of the DNMT1 gene [28].

Furthermore, miR-143 regulates DNMT3a in CRC cells, whereas miR-148a and miR-148b represses Dnmt3b expression in mouse cells through binding to a highly conserved sequence in its coding region rather than the 3′ UTR [29,30]. Benetti et al. [31] proposed a new regulatory pathway for DNA methylation involving the mammalian miR-290 cluster (miR-290, miR-291-3p, miR-291-5p, miR-292-3p, miR-292-5p, miR-293, miR-294 and miR-295) as an important regulator of Rbl2, which in turn acts as a transcriptional repressor of Dnmt3a and Dnmt3b causing hypomethylation in the genome, especially in the telomeres. The whole cluster is shown to be down-regulated in Dicer-null cells in mouse, whereas Rbl2 is increased in expression leading to repression of Dnmt3a and Dnmt3b causing DNA methylation defects [31]. However, the

Table 3. Overview of the miRNAs that regulate the enzymes playing major roles in epigenetic processes

miRNA	Epigenetic mechanism regulated	Cell type/disease	Target	Mechanism of action	Consequence	Reference
miR-29a	*De novo* DNA methylation	Non-small cell carcinoma of the lung	DNMT3a	Up-regulation distrupts *de novo* methylation	Apoptosis of cancer cells, inhibition of cancer growth	[27]
miR-29b			DNMT3b			
miR-29c						
miR-29b	Global methylation	AML	SP1	Silences DNMT1	Global hypomethylation	[28]
miR-143	DNA methylation	CRC	DNMT3a	Inversely correlated with DNMT3A	Decreased tumour cell growth and soft-agar colony formation	[29]
miR-148a	*De novo* DNA methylation	Testicular germ cell tumour cells	DNMT3b1	Translational repression, mRNA degradation		[30]
miR-148b						
miR-290 cluster (miR-290, miR-291-3p miR-291-5p, miR-292-3p, miR-292-5p, miR-293, miR-294 miR-295)	*De novo* DNA methylation	Mouse embryonic stem cell	RBL2	Regulates Rbl2, a transcriptional repressor of Dnmt3a and Dnmt3b	Down-regulation results in increased telomere recombination, aberrant telomere elongation	[31]
	No effect	Human embryonic kidney cells	No regulatory effect on methylation in human cells	Species and cell type specific		[32]
miR-206	Histone deacethylation	Mouse skeletal muscles	HDAC4	Translational inhibition	Modifier of ALS pathogenesis	[62]

(*Continued*)

Table 3. (*Continued*)

miR-140	Histone deacetylation	Mouse cartilage cells	HDAC4	Suppresion of HDAC4	Differentiation	[35]
miR-1	Histone deacethylation	Skeletal muscle tissue	HDAC4	Suppresion of HDAC4		[34]
miR-1 miR-499	Histone deacethylation	Human-derived cardiomyocyte progenitor cells	HDAC4 SOX6		Reduced proliferation, enhanced differentiation into cardiomyocytes	[37]
miR-449a	Histone deacethylation	Prostate cancer cells	HDAC1	Directly targets and represses HDAC1	Cell-cycle arrest apoptosis	[38]
miR-101	Histone methylation	Prostate and bladder cancer	EZH2	Targets EZH2 which is the catalytic subunit of the Polycomb repressive complex 2 responsible for histone H3 lys 27 trimethylation, a mark of epigenetic repression	Inhibition of cancer formation	[39,40]
miR-2861	Histone deacetylation	Primary mouse osteoblasts	HDAC5	Repression of histone deacetylase 5	Promotes osteoblast differentiation	[63]

regulatory effect of the miR-290 cluster on methylation cannot be shown in DICER-knockdown human embryonic kidney cells. This indicates that the miR-290 clusters' effect on DNMTs could be cell-type- or species-specific [32]. Recently, a completely new mechanism was suggested for regulation of gene expression by miRNAs in moss. Khraiwesh et al. propose that initiation of epigenetic silencing by DNA methylation is regulated according to the ratio of the miRNA and its target mRNA [33].

miRNAs also regulate the expression of HDACs and HMTs. HDAC4 is shown to be a direct target of miR-1 and miR-140 [34,35]. A new miRNA HDAC4 regulatory mechanism has been revealed in ALS (amyotrophic lateral sclerosis) which is the most common adult motor neuron disease. miR-206 is shown to delay the progression of ALS, and HDAC4 is both computationally and experimentally shown to be a target of miR-206. Interestingly, in miR-206$^{-/-}$ animals the HDAC4 protein expression is increased in skeletal muscles, whereas *Hdac4* mRNA levels were not changed. This indicated that miR-206 acts upon Hdac4 by translational inhibition rather than at the transcription level [36]. miR-1 and miR-499 are indicated in differentiation of cardiomyocytes, possibly by repression of HDAC4 and SOX6 genes [37].

miR-449a targets HDAC1, which is up-regulated in many cancer forms. miR-449a is down-regulated in cancer, but introduction to prostate cancer cells resulted in cell-cycle arrest and apoptosis [38]. Similarly re-expression of miR-101 in cancer models also resulted in inhibition of cancer formation. miR-101 targets EZH2, the catalytic subunit of the Polycomb repressive complex 2 responsible for histone H3 Lys^{27} trimethylation, a mark of epigenetic repression, and can alter the chromatin structure globally [39,40]. Li et al. [41] identified a new miRNA (miR-2861) in primary mouse osteoblasts that promotes osteoblast differentiation by repressing HDAC5 expression at the post-transcriptional level.

Conclusions

Recently, the molecular mechanisms of epigenetic regulation of miRNA expression and miRNA-mediated control of the epigenetic machinery have attracted much attention, especially in cancer research. By now it is apparent that some miRNA genes are regulated by DNA CpG island hypermethylation and chromatin modifications. Interestingly, these epigenetic marks are potential biomarkers since significant correlations with survival of cancer patients have been found. Likewise, it is also clear that miRNAs regulate various components of the epigenetic machinery and thereby contribute to the regulation of the expression of other genes.

It is essential to explore in more detail this new layer of complexity in gene regulation to improve our understanding of the regulation of the human genome. Importantly, these new insights on the intertwined relationship between miRNA and epigenetics are likely to lead to novel revolutionary anti-cancer therapeutic approaches. Such approaches may be targeting

components of the epigenetic network to cause re-expression of miRNA tumour suppressor genes or directly targeting mature miRNAs or re-expressing miRNAs in order to directly affect target genes and regulate epigenetic feedback loops.

Summary

- *miRNAs are small non-protein coding molecules that regulate more than 30% of the protein coding genes.*
- *miRNAs play an important role in many biological processes such as differentiation, organ development and proliferation.*
- *In cancer and some other diseases such as diabetes, neurological and cardiac diseases, a perturbed miRNA expression is found in the relevant tissues.*
- *Some miRNAs are regulated by epigenetic mechanisms, especially by methylation.*
- *Methylation status of some miRNA genes correlates with survival of cancer patients.*
- *miRNAs may regulate the epigenetic machinery directly or indirectly by targeting enzymes such as DNMTs or HDACs.*

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